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The Use of Reflectance Spectrophotometry for the Assay of Raw Meat Pigments

SUMMARY

The literature on reflectance spectrophotometry, as it applies to meat pigments, is critically reviewed, and improved methods are suggested for determination of total pigments and of the percent metmyoglobin from reflectance data on raw meat. The suggested method for total pigment was based on reflectivity of the meat samples at 525 $m\mu$, the isobestic point for myoglobin, oxymyoglobin, and metmyoglobin. The reflectivity data, when calculated as the corresponding ratios of the absorption coefficient K to the scattering coefficient S were linearly related to total pigment extract from the meat with acidified acetone. K/S values of pigment-free (peroxide-treated) samples were obtained as a base line. Lowering the pH of the meat decreased the K/S value. This was attributed to changes in texture which increased S . Metmyoglobin was determined from the ratio K/S 572 $m\mu$ / K/S 525 $m\mu$. Limiting values for the ratio were established for meat containing 100% and 0% metmyoglobin, and a linear relation was assumed between the ratios and intermediate amounts of metmyoglobin.

INTRODUCTION

To follow rapid changes in pigment composition of fresh meats, it is highly desirable that measurements be made directly on the samples, without first extracting the meat pigments. Extraction procedures are cumbersome, destroy the sample analyzed, and are likely to result in changes in the relative proportions of the three pigments, myoglobin, oxymyoglobin, and metmyoglobin. The use of reflectance spectrophotometry for quantitative measurements of pigments in solid foods is still in a rather rudimentary stage; nevertheless, this appears to be the most promising tool available for such studies. An analysis of the literature on reflectance spectrophotometry and its application to the meat situation leads us to emphasize the following points as a basis for improving current procedures.

The layer of meat used for reflectance studies must be sufficiently thick that no change in reflectance occurs upon further increasing the thickness. The reflectance of such infinitely thick samples has been termed "reflectivity" and given the symbol R_∞ in standard treatments of opaque-colored materials (Judd and Wyszecki, 1963). Whether or not a given thickness of meat meets this condition can be determined most easily by measuring its reflectivity first against a white and then against a black background. There should be no difference in the two readings in any region of the spectrum.

Absorption peaks for the three meat pigments occur at the same wave lengths in reflectance studies on meat as in transmission studies with the pigments in solution. Naughton *et al.* (1957) called attention to the similarity in general form of the absorption curves of meat and of pigment solutions. However, as compared to clear solutions, the spectra of colored solutions in turbid media tend to be less definitive. Absorption peaks are partially flattened and valleys filled in (Butler, 1962).

Isobestic points are also identical in both situations. The isobestic points of myoglobin and metmyoglobin in ground beef are clearly shown (Stewart *et al.*, 1965) and coincide with isobestic points of the same pigments in pure solutions taken from the literature. Only one isobestic point, at 525 $m\mu$, is common to all three pigments in the visible range.

The curves shown were obtained without removing the meat sample from its position on the spectrophotometer. Considerable fluctuations in absorbance at 525 $m\mu$, and consequently in apparent isobestic points, are obtained when one works with different portions of the same lot of ground meat, or even the same portion after removing from the spectrophotometer and repacking. The lack of homogeneity of packed samples is undoubtedly a large factor in lowering the pre-

cision obtainable with reflectance studies on meat.

Although the spectra are qualitatively similar, quantitative evaluations of meat pigments from R_∞ data present unsolved problems. Earlier studies have attempted to use absorbance, i.e., the logarithmic function of "reflectivity" in percent ($2 - \log R_\infty$) in the same way that absorbance data are used in the analysis of solutions by application of Beer's law. Naughton *et al.* (1957) presented data which seemed to show a linear relationship between absorbance and concentration of copper sulfate solutions on crystalline alumina. However, their observations extended over only a very narrow absorbance range. Naughton *et al.* (1958), working with tuna fish, and Erdman and Watts (1957), with cured meats, used absorbance ratios from reflectance studies for rough determinations of proportion of ferric to ferrous pigments.

These studies failed to take into account the fact that a fraction of the incident light at each wave length is scattered in the opaque sample. The pigment solution surrounds a matrix of meat solids. The proportion of light absorbed by the pigment to that scattered by the matrix decreases with increasing R_∞ .

The ratio of the absorption coefficient (K) to the scattering coefficient (S) varies with the total light reflected according to the expression:

$$K/S = \frac{(1 - R_\infty)^2}{2 R_\infty}$$

The derivation of this Kubelka-Munk equation, the tabulated values for K/S at all values of R_∞ , and some examples of the usefulness of this expression in solving problems of matching colors in paints, papers, and textiles are described by Judd and Wyszecki (1963). It should be noted that the value of K , the absorption coefficient, is not obtained from R_∞ measurements alone. To determine K , it is necessary to first compute S , which involves measurements either of the transmittance of layers of known thickness, or of the reflectance of layers of known thickness against backgrounds of known reflectance. This has not been done for meat. If it could be assumed that S is reasonably constant under a given set of experimental conditions,

then K/S values should be proportional to the amount of pigment and should be usable in much the same way that absorbance values are used in the analysis of clear solutions by transmitted light. However, it might be expected that differences in structure of the meat would change the scattering coefficient S , and consequently the K/S value, for a given pigment concentration.

Wodicka (1956) first called attention to the necessity of using K/S values rather than the absorbances in studying the pigment of cured meat. He did not attempt to make quantitative measurements of the meat pigments.

Dean and Ball (1960) employed ratios of K/S values at several wave lengths to estimate the proportion of myoglobin, oxymyoglobin, and metmyoglobin in raw meats. In setting up their ratios, however, they followed Broumand *et al.* (1958) in selecting wave lengths which would not be expected to give a linear relationship between the ratio and the proportion of pigments even with solutions of pure pigments. They then made the assumption that various proportions of the pigments in meat would give the same numerical values for the K/S ratios as those obtained for absorbance ratios of the pure pigments in solution, previously calculated by Broumand *et al.* (1958).

There does not seem to be a good theoretical or experimental basis for this assumption. It must be remembered that absorption of light by meats is only partially due to the heme pigments. Meat in which the heme pigments have been completely destroyed also absorbs and scatters light throughout the visible region. This situation is analogous to that of dyeing fibers—the total K/S values for the dyed fibers must be corrected for the K/S value of the undyed fiber to arrive at useful data for dye concentrations (Mersereau and Rainard, 1951). No absorbance data are available in the literature on pigment-free raw meats.

In view of these considerations, the experimental work reported here was undertaken in an effort to refine the reflectance method as a measure both of total pigment and of the proportion of metmyoglobin to ferrous pigments (myoglobin plus oxymyoglobin).

MATERIALS AND METHODS

Preparation of meat for spectral analysis.

Locally purchased cuts of beef and pork were trimmed of any external fat, then ground twice with the grinder attachment to a Kitchen Aid Mixer, Model 3-C. All of the grinder surfaces in contact with meat were either aluminum or stainless steel.

To study the spectral characteristics of pigment-free meat, 12.5 ml of 30% hydrogen peroxide and 2.5 ml of water were added to 50-g samples of the ground meat. Preliminary experiments established that this rather large amount of peroxide was necessary to bleach the heme pigments, since much of the added peroxide was decomposed by catalase in the tissues. The decomposition was accompanied by a large evolution of gas, which was allowed to escape from the meat by stirring.

The meat pigment could be converted entirely to reduced myoglobin by the addition of 0.01 to 0.02 g of sodium hydrosulfite to 50 g of meat to which had been added 15 ml of water. Once the meat and the reducing agent had been mixed, the typical absorption spectrum of reduced myoglobin persisted for at least 25 min.

For conversion to metmyoglobin, potassium ferricyanide (0.05–0.1 g) in aqueous solution was added to 50 g meat. Again the total liquid added was 15 ml. This produced a typical metmyoglobin spectrum if examined immediately, but the ferric pigment is reduced more or less rapidly in different samples of meat (Stewart *et al.*, 1965).

Spectral analysis. All spectral curves were obtained on a Bausch and Lomb recording Spectrophotometer, Model 505, with reflectance attachment. The prepared meat samples were placed in aluminum cups $\frac{7}{8}$ in. deep and wide enough to cover the sample ports. This thickness of meat was sufficient under all conditions to satisfy the requirements for R_{∞} measurements. The meat was covered with a glass slide which was almost optically clear in the visible region. A similar slide was used to cover the reference standard.

The usual magnesium carbonate reference representing 100% reflectance could not be used conveniently for most meat samples because the absorption of the meat was so great that peaks often exceeded an absorbance of 1 and the scale had to be changed during the recording. For this reason, the meat spectra were actually recorded either against a hydrogen-peroxide-bleached sample of the same meat or, in later experiments, against a piece of gray cardboard. However, the absorbance of the bleached meat or cardboard against the magnesium carbonate standard was always added to that of the meat at each wave length under consideration so that the values presented throughout the paper

represent the total absorption of the meat against magnesium carbonate.

The spectrophotometer was set to record in absorbance units rather than percent reflectance. To obtain K/S values, the readings were first converted to R_{∞} from standard tables relating absorbance to percent transmittance. The K/S value corresponding to each R_{∞} was then read from Table D in the appendix of Judd and Wyszecki (1963).

RESULTS AND DISCUSSION

Spectra of bleached meat. Spectra were obtained for 16 samples of bleached meat from rib, chuck, and round of beef and pork loin. All were qualitatively similar, showing no peaks but a gradual increase in absorption from longer to shorter wave lengths in the spectral region between 700 and 450 $m\mu$. The absorbance at 525 $m\mu$ of the sixteen samples varied from 0.18 to 0.47, averaging 0.33. A typical curve having an absorbance of 0.30 at 525 $m\mu$, had values of 0.20 at 700 $m\mu$ and 0.41 at 450 $m\mu$. Although the absorbance of pork samples averaged less than that of beef, there were not sufficient samples to be sure that this was a consistent or significant difference. Considerable variation could be obtained even in the spectra of different portions of the same lot of meat, probably due largely to differences in air trapped in the samples. Bleached cooked meat showed spectra of the same general shape, but always with higher absorbances than the corresponding raw samples.

K/S at 525 $m\mu$ as a measure of total pigment. Since 525 $m\mu$ is isobestic for all three meat pigments, the reflectivity values at this wave length, expressed as the ratio K/S , might be expected to show some correlation with the total amount of heme pigments in the meat.

The total pigment from a number of samples of beef and pork was extracted with acidified acetone according to the method of Hornsey (1956). The assays were made in duplicate and averaged. The total pigment, expressed as parts per million of hematin, was plotted (Fig. 1) against the K/S values obtained from spectral curves of meat samples to which 15 ml water had been added to 50 g meat. This large amount of water was necessary in the original experimental work

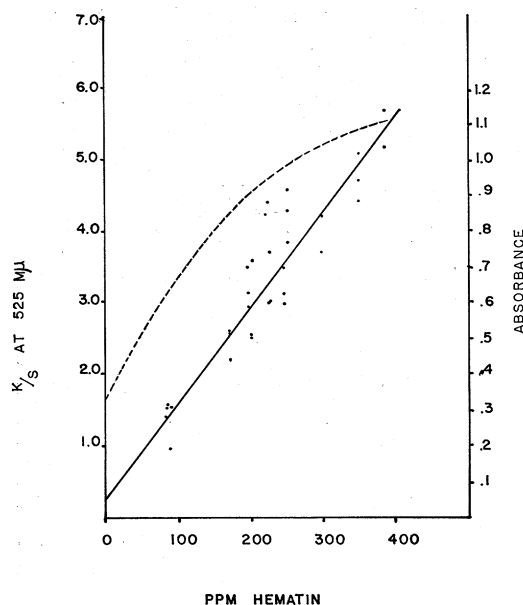


Fig. 1. Hematin concentration of pork and beef samples as related to K/S values at 525 $m\mu$ (—) and to absorbance values at 525 $m\mu$ (---).

to accommodate the amounts of peroxide, ferricyanide, etc., used to modify the pigments. However, it made the meat samples too soft to handle easily, and certainly would not be recommended for routine use. Some of the meat samples had been completely reduced, others oxidized; and in still others the pigment was largely oxymyoglobin. The distribution of points on the plot was not related to the oxidation state of the pigment; therefore, the pigment forms are not differentiated in Fig. 1. The K/S value plotted at zero hematin concentration was that obtained from the average absorbance of the sixteen bleached samples (absorbance 0.33, $K/S = 0.30$). Shown on the same figure (without experimental points) is the curve drawn through the same data when plotted as absorbance rather than K/S values.

Several conclusions can be drawn from these data. There is a positive correlation between the readings at 525 $m\mu$ and total pigment. Expressed as absorbance values, the relation is definitely nonlinear. When plotted as K/S , a straight line appears to best fit the data. The rather large scattering of experimental points is evidence of the lack of precision of the method. It must be re-

membered, however, that the samples represent a number of cuts, both of beef and pork, varying in pH, fat, connective tissue, etc., as well as in pigment.

It might be expected that any treatment or condition which significantly affects the texture of meat might also affect the scattering coefficient S and thus the relation between the K/S values at 525 and the actual concentration of pigment present. The large effect of changes of pH on meat texture is well known (Bate-Smith, 1948). As the pH is lowered, meat fibrils tend to shrink and scatter light. Meat of the same actual pigment content appears to be lighter in color.

Table 1. Effect of pH adjustment on K/S at 525 $m\mu$.

pH	K/S 525 $m\mu$
5.1	2.37
5.45	3.13
5.8	3.95
6.5	4.42
7.1	4.31

Table 1 shows the effect of varying the pH of a single sample of ground beef on the K/S values at 525 $m\mu$. The original pH of the sample was 5.8; it was treated with 5N NaOH or 1N HCl to give the pH values recorded in Table 1. All samples had the same amount of total liquid added (5 ml to 100 g). Obviously, if there are large fluctuations in pH, unadjusted K/S values are not a useful measure of total pigment. Since pH measurements were made on only a few of the samples analyzed for total pigment (Fig. 1), it is not possible to say to what extent variations in pH were responsible for the scattering of experimental values. Normal variations in pH are not as wide as those shown in Table 1. The range in 20 samples of rib eye from different animals was from pH 5.5 to 5.8. Swift *et al.* (1960) reported that the pH of eight different muscles in one animal varied from 5.53 to 6.09. It is possible that a correction factor, based on more extensive data of the kind shown in Table 1, could be applied to adjust K/S to the expected value at some arbitrarily chosen pH.

In spite of the many possible sources of error, the method appears to offer sufficient

promise to warrant further testing on untreated ground meat or surfaces of whole cuts. It is possible that the precision could be improved if the method were applied to more uniform material, or if corrections were applied for pH variations.

Proportion of metmyoglobin to ferrous pigments. Examination of the absorption spectra of identical concentrations of the three meat pigments, reproduced from Bowen's original data by Dean and Ball (1960) and measured independently by Taylor (1961), show, in addition to the isobestic point for all three pigments at 525 $m\mu$, an isobestic point for myoglobin and oxymyoglobin at 572 or 573 $m\mu$. At these latter wave lengths, the absorption of metmyoglobin is very much less than that of the two ferrous pigments. The ratio of absorbances at 572/525, when plotted against percent of the total pigment as metmyoglobin, gives a straight line, in contrast to the curve obtained by plotting a similar ratio at 507/573, as was done by Broumand *et al.* (1958).

While it is not experimentally feasible to work with known mixtures of pigments in meats, it is easily possible to examine portions of the same meat in which the pigments have been converted entirely to metmyoglobin on the one hand or to reduced myoglobin on the other by treatment with ferri-cyanide and hydrosulfite, respectively.

Table 2. Ratios of K/S at 572 $m\mu$ to K/S at 525 $m\mu$ for known pigments.

Pigment	No. of Samples	Ratio		Standard deviation
		Range	Av.	
Metmyoglobin	19	0.51-0.65	0.56	.04
Reduced myoglobin	20	1.13-1.61	1.40	.16

Table 2 summarizes K/S ratios at 572 $m\mu$ /525 $m\mu$ calculated from the reflectance curves of a number of samples of ground beef. Again, there is a considerable spread of experimental points around the average values. The average values for the K/S ratios of reduced myoglobin are practically identical with the absorbance ratios of pure pigment solutions whereas the metmyoglobin K/S ratios are definitely higher than ab-

sorbance figures. (calculating from Bowen's data, the ratios at 572 $m\mu$ /525 $m\mu$ are approximately 1.38 for the ferrous pigments and 0.40 for metmyoglobin; from Taylor's data, the corresponding ratios are 1.45 and 0.49). It should be emphasized that any agreement between the numerical values for the K/S and absorbance ratios is fortuitous. If the wave lengths selected for comparison had been nearer the maxima and minima of the absorption curves, the discrepancies would be much greater.

The K/S ratios for the same meat samples at the wave lengths used by Dean and Ball (1960), i.e., 507 $m\mu$ /573 $m\mu$, gave an average value for reduced myoglobin of 0.50 (range 0.41-0.62). This average is close to the corresponding absorbance ratio, approximately 0.54, of reduced myoglobin in solution. On the other hand, the average K/S ratio for metmyoglobin at these wave lengths (close to the peak and valley of the metmyoglobin curve) was 1.86 (range 1.35-2.20) as compared to 3.3 for 100% metmyoglobin in solution. This large discrepancy emphasizes the danger of using absorbance ratios obtained on pure pigment solutions to predict the proportion of pigments corresponding to K/S ratios in meat.

In using the K/S ratios at 572 $m\mu$ /525 $m\mu$, a linear relation is assumed between the ratios and the percent metmyoglobin in the meat sample (Fig. 2). A linear relation would be expected at this choice of wave

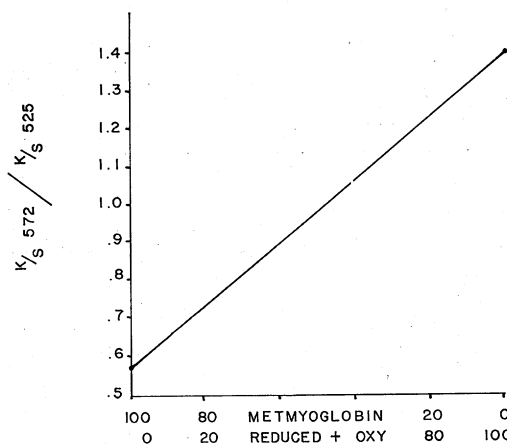


Fig. 2. Assumed linearity between limiting K/S ratios at 572 $m\mu$ /525 $m\mu$ for 0 and 100% metmyoglobin.

lengths, although the relationship cannot be experimentally verified in meats, nor have we been able to find in the literature a similar treatment of analogous situations. Snyder (1964) reports absorbance data on known mixtures of two heme pigments in milk. A comparison of K/S ratios in such a system would be enlightening.

In using this assumed relationship to determine metmyoglobin in meat, the wide spread of experimental points which have been averaged to obtain the limiting values in Fig. 2 must be kept in mind. Again, more precise results might be obtained on individual samples of meat by determining the limiting K/S ratios for 0 and 100% metmyoglobin for this particular meat, and calculating intermediate states of pigment reduction from these rather than from the averages of Fig. 2.

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